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Angiotensin I-converting enzyme in isolated human glomeruli

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Angiotensin I-converting enzyme (ACE) activity was measured with hippurylhistidylleucine as a substrate in isolated human glomeruli. The mean level was 2.2±0.47 mIU/mg glomerular protein. S9780, a newly designed competitive inhibitor of ACE, inhibited this activity by 85% at 0.3 μM. [^{3}H]S9780 specifically bound to isolated human glomeruli. The K_d value and the number of sites were 23 nM and 83 fmol/mg, respectively. The prodrug, S9490, and Captopril were less potent than S9780 in displacing [^{3}H]S9780 from its binding sites. Angiotensin I had no effect. Binding of [^{3}H]S9780 was inhibited after preincubation of the glomeruli with a specific polyclonal anti-human ACE antibody. These results demonstrate that ACE is present in human adult glomeruli.

Angiotensin I-converting enzyme; Enzyme inhibitor; (Glomerulus)

1. INTRODUCTION

The presence of angiotensin I-converting enzyme or kininase II (ACE; EC 3.4.15.1) in human kidney [1-3], and especially in the brush border of the proximal tubule [4], has been extensively demonstrated. Localization of the enzyme in the glomerular capillaries is more controversial. Immuno-histochemical techniques, using specific antibodies to human renal ACE, did not detect any labelling in the glomerular capillaries examined in sections of human adult kidneys [5]. Only endothelial cells in glomeruli of fetal kidneys were lined with the reaction product [6]. ACE activity was also present in cultured human glomerular endothelial cells [7]. Results in other species are scarce. ACE activity was demonstrated in rabbit freshly isolated glomeruli [8] and in rat cultured glomerular endothelial cells [9]. In the present paper we report the presence of ACE in human glomeruli. The enzyme was detected by its biological activity and the specific binding of a ^{3}H-labelled ACE inhibitor, S9780.

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2. MATERIALS AND METHODS

S9780 (Perindopril), its prodrug S9490 (Perindoprilate) and [^{3}H]S9780 were donated by the Institut de Recherches Servier (Neuilly, France). The labelled molecule was prepared by the Centre d'Etudes Nucléaires (Gif-sur-Yvette, France). Its specific activity determined by mass spectrometry after purification by high performance liquid chromatography was 55 Ci/mmol. S9490 and its diacid active form, S9780, are competitive inhibitors of ACE synthesized by Vincent et al. [10]. Captopril was a gift from Squibb (Neuilly, France). Angiotensin I (A I) and hippurylhistidylleucine were purchased from Sigma (St. Louis, MO).

2.1. *Glomerular preparations*

Human glomeruli were isolated according to

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[11] from adult kidneys obtained at nephrectomy or judged unsuitable for transplantation. They were checked for purity under light microscopy. All the preparations studied were devoid of any tubular contamination.

Human glomerular mesangial cells were cultured as described in [12] in Waymouth's medium supplemented with 10% fetal bovine serum, 100 mU/ml penicillin and 100 μg/ml streptomycin. They were studied after 4 passages. Dissociated cells were removed from the Petri dishes using a rubber policeman. Then, the cells were washed and suspended in 20 mM Tris-HCl buffer, pH 7.5, containing 135 mM NaCl, 10 mM KCl, 10 mM $NaCH_3COO$ and 5 mM glucose (buffer A).

Glomerular or cellular protein concentration was determined according to [13].

2.2. *ACE activity assay*

ACE activity was measured according to [14] with the modification described in [15] in isolated glomeruli which had been homogenized by sonication. In this technique, hippuric acid formed by hydrolysis of hippurylhistidylleucine is measured spectrophotometrically at 228 nm in the aqueous phase obtained after a differential extraction procedure. The incubation medium was first extracted with ethylacetate. Then, the ethylacetate extract was evaporated gently under a stream of air and re-extracted with a two phase system (1 M NaCl:petroleum benzine; 3:2, v/v). In some samples, the glomerular homogenate was incubated with 0.3 μM S9780. One unit of ACE activity is defined as the amount of enzyme able to hydrolyze 1 μmol substrate per min.

2.3. *Binding studies*

Glomeruli or glomerular mesangial cells (50 μg protein) were added to tubes containing approx. 10 nM [^{3}H]S9780 in buffer A. Incubation was performed at 22°C under continuous gentle shaking usually for 20 min. Non-specific binding was measured in parallel in tubes containing an additional amount of unlabelled S9780 (10 μM). Bound and free radioactivities were separated by filtration through a cellulose nitrate filter (Whatman, Maidstone, England; 0.45 μM). The filter was dried after 3 successive washings with ice-cold 10 mM Tris-HCl buffer, pH 7.4, and solubilized in 8 ml of ACS II (Amersham, England). ^{3}H radioactivity was counted with 43% efficiency using a liquid scintillation spectrometer (model Rackbeta 1211; LKB, Malmö, Sweden). The blank value corresponding to the percentage of radioactivity bound to the filter in the absence of glomeruli or glomerular cells was always lower than 0.1% of total radioactivity added. Specific binding was obtained by subtraction, from total binding, of binding in the presence of 10 μM unlabelled drug. It was expressed as fmol of S9780 bound per mg protein.

[^{3}H]S9780 binding to human glomeruli was also measured following a 1-h preincubation at 37°C with a specific polyclonal anti-human ACE antibody at 1/500 final dilution. The results were compared with those obtained in parallel in the presence of preimmune rabbit serum at the same dilution. The anti-ACE antibody was a gift of Doctor Alhenc-Gelas (Paris). Its characteristics have previously been reported [16].

3. RESULTS

3.1. *ACE activity in human glomeruli*

ACE activity in homogenized human glomeruli was 2.2 ± 0.47 mIU/mg (table 1). ACE activity

Table 1

Angiotensin I-converting enzyme activity in human glomeruli

Preparations studied (code number)	Angiotensin I-converting enzyme activity (mIU/mg)	Percentage of inhibition by S9780
2	1.73	84
3	2.87	93
4	1.13	88
5	0.93	80
30	0.78	89
35	4.63	89
44	2.07	92
45	5.23	not measured
49	1.48	74
50	1.17	74
Mean ± SE	2.2 ± 0.47	84.8 ± 2.3

Preparations 2, 3, 4 and 5 were from kidneys obtained at nephrectomy. Preparations 30, 35, 44, 45, 49, 50 were from kidneys judged unsuitable for transplantation. S9780 was used at 0.3 μM

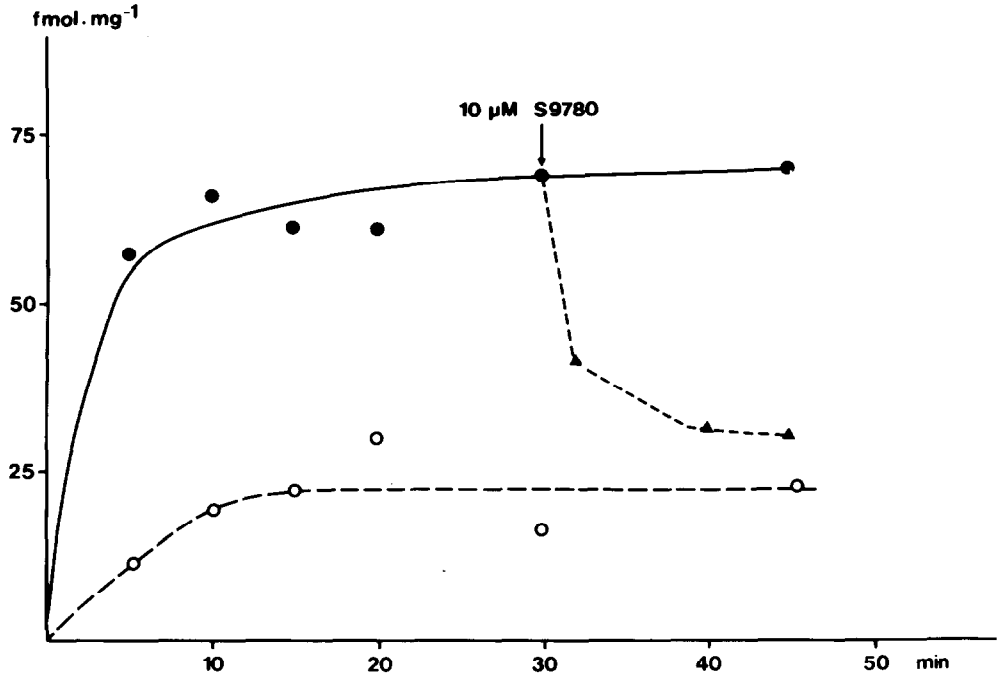

Fig.1. Time-course of total (●) and non-specific (○) binding of [^{3}H]S9780 to human glomeruli. Concentration of [^{3}H]-S9780 was 10 nM. Dissociation of the [^{3}H]S9780-glomerulus complex (▲) was obtained after addition of 10 μM unlabelled S9780 after equilibrium had been reached. Each point is the mean of duplicates.

was also measured in the whole renal cortex homogenate in some of the samples. It was approx. 4-times higher (8.1 ± 2.8 mIU/mg) than in the corresponding glomeruli (1.9 ± 0.5 mIU/mg). Glomerular ACE activity was strongly inhibited (84.8 ± 2.3%) in the presence of 0.3 μM S9780.

3.2. *Binding studies*

[^{3}H]S9780 binding to human glomeruli was measured as a function of time (fig.1). Equilibrium was reached after 15 min and persisted until 50 min. Specific binding represented approx. 70% of total binding. Addition of an excess of the unlabelled drug (10 μM) at equilibrium produced displacement of [^{3}H]S9780 from its binding sites as shown by the rapid decrease of bound radioactivity which reached the value of non-specific binding within 15 min. There was a linear relationship ($r = 0.88$; $p < 0.01$) between [^{3}H]S9780 bound and the amount of glomerular protein in the range tested (0–120 μg/tube) in the presence of 10 nM [^{3}H]S9780. The slope of the regression line indicated 39 fmol [^{3}H]S9780 bound per mg glomerular protein. When human glomeruli were

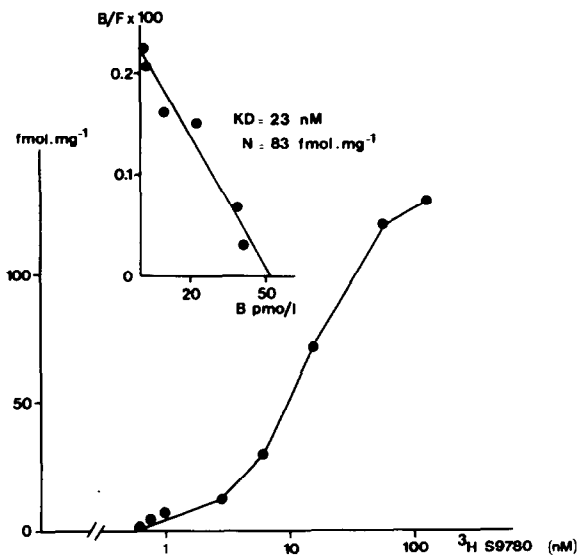

Fig.2. Specific binding of [^{3}H]S9780 to human glomeruli at equilibrium (20 min) as a function of [^{3}H]S9780 concentration (0.6 to 130 nM). Scatchard transformation is given in inset. The corresponding K_d value and number of sites are shown. Each point is the mean of duplicates.

incubated for 20 min with increasing concentrations of [^{3}H]S9780 (0.6–130 nM), the amount of [^{3}H]S9780 specifically bound followed a curvilinear ascending curve (fig.2). No plateau was reached within the range of concentrations used. Scatchard transformation of the data (fig.2, inset) provided a straight line, which suggests the presence of one group of specific binding sites. The apparent K_d value and binding site capacity were 23 nM and 83 fmol/mg glomerular protein, respectively. Dilution of [^{3}H]S9780 (10 nM) with increasing concentrations of unlabelled S9780 from 1 nM to 10 μM decreased the percentage of labelled bound product. Nearly total binding inhibition was obtained at 10 μM and the concentration corresponding to 50% specific binding at zero dose (ED_{50}) was 42 nM. This value is of the same magnitude as the apparent K_d value calculated from the Scatchard plot. Competitive inhibition was also studied in the presence of S9490 which is the prodrug of S9780, Captopril or A I which is the physiological substrate of ACE. ED_{50} were 10 μM and 400 nM for S9490 and Captopril, respectively. No displacement was observed with A I (fig.3).

Specific binding of [^{3}H]S9780 to isolated glomeruli was inhibited by 41% after preincubation of the glomeruli in the presence of a polyclonal anti-human ACE antiserum at 1/500 final dilution (39.9 ± 7.3 vs 67.3 ± 3.7 fmol/mg; $n = 7$; $p < 0.01$). In contrast, there was no inhibition of binding after preincubation with preimmune serum at the same dilution (62.3 ± 4.0 fmol/mg).

No specific binding of [^{3}H]S9780 to cultured human mesangial cells was observed.

4. DISCUSSION

The results of this study strongly suggest the

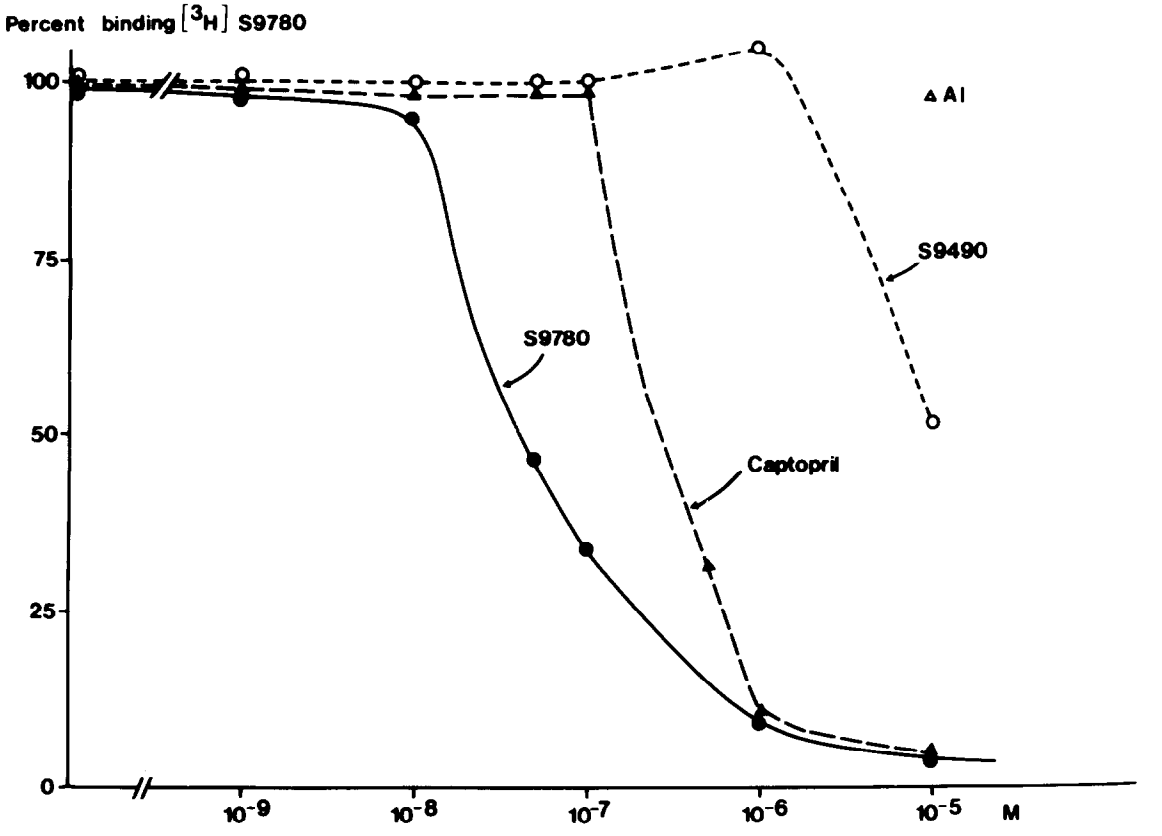

Fig.3. Competitive inhibition of [^{3}H]S9780 binding to human glomeruli in the presence of increasing concentrations of unlabelled S9780, S9490 (prodrug) and Captopril. The effect of 10 μM angiotensin I (A I) was also studied. Each point is the mean of duplicates.

presence of ACE in human glomeruli. Hydrolysis of hippurylhistidylleucine is likely to reflect ACE activity specifically since it is markedly inhibited at a low concentration (0.3 μM) of an ACE inhibitor, S9780. Furthermore, glomeruli possess binding sites for S9780 which exhibit the admitted characteristics of specificity: equilibrium of binding with time, reversibility of binding by addition of the unlabelled drug, competitive inhibition of binding at increasing concentrations of the drug and of parent compounds, high affinity of binding corresponding to the active concentration of S9780 in human plasma [17]. Finally, binding of [^{3}H]S9780 to human glomeruli was inhibited by an antibody directed against the human ACE. It was not possible to displace [^{3}H]S9780 from its binding sites in the presence of an excess of A I although S9780, like Captopril, is considered as a competitive inhibitor [18]. This suggests that inhibition of [^{3}H]-S9780 binding and enzymatic activity are due to binding to distinct sites. A similar conclusion stemmed from the results of inhibition of [^{3}H]Captopril binding to lung membranes by peptide analog ACE inhibitors [19]. In this study, there was no correlation between inhibition of [^{3}H]Captopril binding and ACE activity. Localization of ACE within the human glomerulus remains uncertain. There was no specific binding of [^{3}H]S9780 to cultured human mesangial cells. One of us (J.-P.M, unpublished) detected a marked labelling of the glomerular mesangium by histoautoradiography after in vivo administration of [^{3}H]S9780 to rabbits. In contrast, Mounier et al. [6], using immunohistochemical techniques, observed that ACE was present on the glomerular endothelial cells of the fetal kidney. These results are in agreement with those of Striker et al. [7] who studied cultured glomerular endothelial cells. Therefore, ACE distribution may be different with the species.

Glomerular ACE has been demonstrated in the rabbit [8] and in the rat [20]. The fact that it was not detected previously in human adult glomeruli [5,6] by immunohistochemistry is probably due to its low concentration in this species. The techniques used in the present study allowed us to get over this difficulty.

The physiological role of glomerular ACE is probably related to the intraglomerular formation of angiotensin II. Renin is synthesized by the differentiated epithelioid cells of the glomerular afferent arterioles but also, within the glomerulus, by the mesangial cells [21]. Angiotensinogen, which serves as a substrate for the formation of A I, is present in the plasma and is also synthesized in the renal cortex [22]. Therefore, all conditions are met for intraglomerular generation of A II. Glomerular mesangial cells and the glomerular arterioles are the target of this hormone which produces essentially an increase in the glomerular intracapillary pressure and a decrease in the effective surface of filtration [23]. Furthermore, it becomes increasingly evident that intrarenally generated A II plays a major role in a number of conditions such as chronic salt depletion, adrenergic nerve stimulation or nephrotoxic acute renal failure [24]. Demonstration of ACE in glomeruli is a supplementary reason for using ACE inhibitors in the control of local A II generation.

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